column is a 125 mm × 4 mm × 5 μm Lichrosphere cyano (these are typical column dimensions for a standard HPLC separation). The sample is drawn directly from a titre plate and injected. The modifier is programmed from 15% to 65% at 45% min^{-1}. The analysis takes 1.2 min to complete and an additional 0.68 min to re-equilibrate. Consequently, the system is able to run over 760 samples per day. On a similar column by reversed-phase HPLC, the system is capable of two to three runs per hour or at best, 72 samples per day.

Note that the chromatogram shows several peaks in the sample. Since the goal of this synthesis was a single component, there are some obvious complications. With the column used, the system has sufficient peak capacity to separate the seven components identified. For a similar speed of analysis by HPLC, the column would have to be substantially shorter or have much smaller particles. In either case, the peak capacity would not be the same at the SFC separation. As a result, the HPLC system may be unable to resolve all of the peaks. The speed and efficiency of SFC is better suited to these high volume analyses than HPLC.

Summary

The rapid analysis speed, complementary selectivity, and achievable high efficiency of SFC make it a valuable technique for pharmaceutical analysis. Because of its normal-phase-like retention mechanism, SFC affords users of reversed-phase HPLC an orthogonal separation system. In addition, chiral separations are clearly a strong application for SFC. The renewed interest in SFC for laboratory-scale purifications and combinatorial screening has breathed new life into this technique.

A valid point raised by Wilson *et al.* is that the current literature still demonstrates the potential of SFC rather than fully developed methods. This situation has begun to change. Although the literature may not reflect it yet, more and more pharmaceutical workers are using SFC as a routine technique.

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Thin-Layer (Planar) Chromatography

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In 1938, Izmailov and Schraiber used aluminium-coated plates to separate coloured plant extracts. Further development of thin-layer chromatography (TLC) as a semiquantitative analytical technique and a tool for identity testing took place in the mid-1950s, especially by Stahl. Soon after this pioneering work, TLC found its way into pharmaceutical analysis.

Surveys of the analytical literature from the late 1980s to the mid-1990s show that approximately 25–30% of all articles published in the field of TLC described pharmaceutical applications. These figures may give some idea about the status of TLC in pharmaceutical analyses.

The rapid development of liquid chromatography (LC, especially high performance liquid chromatography (HPLC)) in the mid-1970s has made LC the predominant analytical method in pharmaceutical analysis. Nevertheless, even today TLC must be considered the most widely used pharmacopoeial chromatographic technique.

TLC in the Pharmacopoeias

An early example of pharmacopoeial use of TLC (**Table 1**) was for impurity testing of corticosteroids in the *British Pharmacopoeia* (BP) addendum 1966 and as a general method featured in the BP of 1968. A typical specification for an active pharmaceutical ingredient until then would require verification of identity and then rely on a usually nonspecific assay supplemented by traditional limit tests, e.g. for heavy metals and chloride. For most organic substances a reasonably sharp melting point was a crude but generally accepted measure of purity. The introduction of TLC to the BP 1968 had great expectations of this technique, as it refers to 'greatly increased

Table 1 TLC in the pharmacopoeias

1964	DAB 7 – GDR: general method
1966	*BP* addendum: corticosteroids
1968	*BP*: general method
1980	DAB 8 – add. – FRG: general method
1985	DAB 9 – FRG: 'quantitative' determination of glycyrrhizic acid in liquorice root
1998	DAB – 1998 monograph 'soja lecithin': assay of phosphatidylcholine via quantitative TLC
1999	EP addendum 1999: revised general monograph TLC including quantitative TLC

DAB, German Pharmacopoeia; FRG, Federal Republic of Germany; GDR, German Democratic Republic; EP, European Pharmacopoeia.

emphasis placed on detection and control of impurities [from] manufacture or degradation … made possible by the rapid development of TLC as a reliable means of detecting and assessing small quantities'.

In its basic form, as developed then, TLC is simple, rapid, robust and inexpensive and can be performed in nearly every analytical environment. Therefore it is still widely used in national and international pharmacopoeias for identity testing of active pharmaceutical ingredients and excipients, especially of compounds of natural origin like plant extracts or herbal preparations. In its semiquantitative mode, where spots of reference test solutions are usually visually matched against the impurity spots in the chromatogram of the undiluted test sample, it is still the widest used chromatographic technique to control impurities in either active pharmaceutical ingredients or drug products.

High performance TLC (HPTLC), with layers composed of particles with smaller diameters and narrow particle size distributions, gives greater separation efficiency and improved detection limits. Automated scanning densitometers have led to instrumental quantitative TLC, but neither of these developments has found its way into the national and international pharmacopoeias until recently.

Today, most of the TLC procedures included in pharmacopoeial monographs must be considered to represent an obsolete form of this technique, leading to the misconception that TLC *per se* is merely a qualitative or at best semiquantitative technique lacking in accuracy and sensitivity (**Figure 1**).

But even when performed under state-of-the-art conditions, separation power and sensitivity are generally lower for TLC than for HPLC. As a consequence, there is a general shift away from semiquantitative TLC to quantitative HPLC for the control of impurities in new or revised pharmacopoeial monographs. This development is especially justified by the requirement of the guidelines of the International Conference on Harmonization (ICH), to control unknown impurities down to a threshold limit of 0.1% – originally intended for new chemical entities only – but today also applied to well-known active pharmaceutical ingredients, listed in pharmacopoeias. This general shift away from TLC to HPLC for the control of impurities and related substances must be considered irreversible.

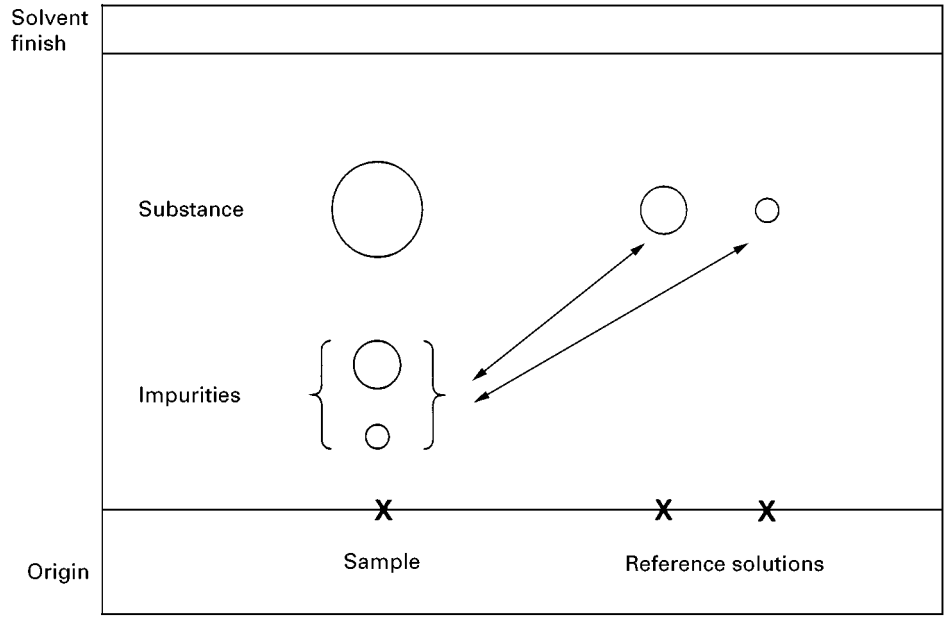

Figure 1 Pharmacopoeial use of TLC: testing for related substances.

Table 2 Quantitative TLC in the general monograph TLC of the Addendum 1555 of the European Pharmacopoeia: features

Plates	Laboratory-prepared plates abandoned; commerically available plates referred to in reagent section
Performance test	Plates are tested using a mixture of Sudan red, methyl orange, bromocresol and methyl red for separation efficacy and/or with benzoic acid for fluorescence quenching
Preconditioning	Washing or impregnating of plates, if appropriate
Development	Vertical and horizontal development described
Visual estimation	Described for related substances tests and identification
Verification of separation power	Described for related substances tests and identification
Verification of detection power	Described for related substances tests
Quantitative measurement	Described for substances responding to UV-visible irradiation (remission or fluorescence) or containing radionuclides usiing three-point calibration (approx. 80, 100, 120% of expected value) and scanning densitometry
Resolution factor	Results are only valid if the resolution (R_s) between measured peak in the chromatograms is greater than 1.0
Signal-to-noise ratio	Described for determination of the detection limit (DL)

However, the progress in quality of sorbent materials, pre-coated plates and instrumentation that has led to a remarkable improvement in the reliability of instrumental quantitative TLC, has led to its recent introduction as a general pharmacopoeial method. The revised general monograph 2.2.27 *TLC* of the addendum 1999 of the *European Pharmacopoeia* for the first time includes a description of quantitative TLC via automated scanning densitometry and lists the required performance parameters *resolution* and *limit of detection* (**Table 2**).

The first example of the pharmacopoeial use of fully automated quantitative TLC is the assay for phosphatidylcholine in the monograph *Soya Lecithin* of the addendum to the *German Pharmacopoeia DAB* (1998).

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Potential Applications of TLC/HPTLC

For many years TLC was not considered to be a reliable quantitative technique and it was displaced by the techniques of gas chromatography, introduced as a general method in the *BP* of 1968 and, in the mid-1970s by LC, especially HPLC, which today is the most popular technique for assays and purity testing (**Table 3**).

It must be emphasized that only approximately 20% of analytical work in the pharmaceutical industry is controlled by pharmacopoeias. The remaining 80% of analytical problems, such as analytical work in research and development, reaction and process control, stability testing, analytical control of cleaning and its validation may be solved by every available analytical technique, assuming that the corresponding validation and performance data prove that it is suitable for the intended use.

When used with plates containing fluorescence indicators that allow detection of UV-active substances by UV irradiation for identification, the existence of more than 1000 specific derivatization agents represents a major advantage of TLC over other chromatographic techniques.

Hyphenated techniques, or combinations of TLC with spectrometric techniques like Fourier transform near infrared spectroscopy (FT-NIR), Fourier transform infrared spectroscopy (FTIR), diffuse reflection infrared Fourier transformation (DRIFT), Raman/surface enhanced Raman spectroscopy (SERS) or mass spectrometry (MS) with different ionization techniques, are reserved for special applications and are not as commonly used as simple visualization.

For fast, rough quantifications – either in purity and related substance testing or for assays – TLC coupled with visual examination and/or with the emerging technique of video densitometry must be considered the most flexible and economic alternative of all chromatographic techniques.

But TLC is no longer the traditional, uncomplicated but less reliable technique. Reliable, technically mature automated instruments are available for individual steps like sample application, development, derivatization, scanning and quantitation.

For assays and content testing, TLC with scanning densitometry must be considered a realistic alternative to other chromatographic techniques concerning analytes with problematic detection characteristics. Also it is suitable with crude or dirty sample matrices, especially when particulate impurities like plant extracts of finished pharmaceuticals raise problems with other analytical techniques.

Sometimes the weakness of TLC – its lower separation power – can be an advantage: when components consisting of mixtures of oligomers or having different chain length distributions have to be assayed in different matrices.

Table 3 Potential applications of TLC/HPTLC in pharmaceutical analysis

Type of analytical procedure	*Field of application*	*Principle(s)*
Qualitative/identification (API)	Quality control of components, active pharmaceutical ingredients (API), finished pharmaceuticals Single container identification Quality control of herbal/vegetable drugs and fermentation products At-site and port-of-entry testing	Parallel chromatography of sample and reference standard or reference sample, evaluation and comparison of R_F values, spot size and colour (after derivatization)
	Forensic and drug of abuse screening	Evaluation and coomparison of R_F values using different mobile-phase systems with data from libraries, specific derivatization if required, *in situ* or offline spectroscopy of selected spots/bands
Semiquantitative purity test (limit test)	Quality control of coomponents, API, finished pharmaceuticals Quality control of herbal/vegetable drugs and fermentation products At-site and port-of-entry testing: check for adulterations and contaminations	Parallel chromatography of sample and diluted (1 : 50, 1 : 60, 1 : 200) test solution or solutions of potential impurities. Visual match of spot size and intensity (after derivatization if required) with following estimation of impurity/related substances content. Increased reliability and precision using video densitometry.
Semiquantitative assay/content testing	Reaction/process/cleaning control or optimization in: API synthesis Pharmaceutical manufacturing Fermentation Plant/herbal extraction Assay of herbal/vegetable drugs and extracts	Parallel chromatography of reaction mixture or rinse solution and educt/starting materials/previous sample/previous rinse solution. Visual estimation and/or video integration and estimation of process reaction/cleaning progress Parallel chromatography of sample and different concentrations of lead component/reference sample. Visual evaluation and/or video inegration after derivatization if required.
Quantitative assay/content tesing/ impurity/related substances testing	Quality control of APIs and finished drugs Quality control of herbal drugs/extracts and fermentation products Assays and purity testing in stability studies (development, ongoing, follow-up) Bioanalytics: bioavailability studies, pharmacokinetic studies Reaction/process control or optimization: impurity profiles	Parallel chromatography of sample and different concentrations of reference standard and/or dilutions of main component and/or reference sample. Pre- or postchromatographic derivatization if required, preferred: automated spotting, development under controlled conditions, quantitative evalution via calibration function using peak area or peak height after scanning densitometry (or video integration if applicable)

Validation and Performance Characteristics

Like any other analytical technique, TLC used in pharmaceutical quality control or in stability studies and later stages of pharmaceutical development (clinical trial batches) has to be validated in line with the latest guidelines of the ICH. As planar chromatography allows the simultaneous analysis of up to 24 samples on one plate, the time and effort required to validate a procedure are distinctly lower than for other chromatographic techniques.

Performance data verify that, under optimized conditions, quantitative TLC or HPTLC results are comparible to those of LC. A prerequisite is parallel testing of two sample weightings with double-spotting (resulting in four spots for every unknown) and – mandatory according to the monograph in the *European Pharmacopoeia* – a three-point calibration (e.g. 80, 100, 120% of label claim in assays) for linear and preferably four-point calibration for nonlinear calibration models. Except for fluorescence measurements, calibration functions in TLC are generally nonlinear. Narrow specification limits and working

Table 4 Comparison of performance characteristics of TLC, HPTLC and HPLC

Separation power	Technique	Performance
Separation number	TLC	~ 10
	HPTLC	~ 15–20
	HPLC	~ 150
Sensitivity		
Detection limit (UV absorption)	TLC	~ 1–5 μg
	HPTLC	~ 0.2–0.5 μg
	HPLC	~ 0.05–0.3 μg
Precision		
RSD interm. precision (assay)	TLC	~ 1.5–3.0%
	HPTLC	~ 1.0–2.0%
	HPLC	~ 0.8–1.5%

RSD, random standard deviation; interm. precision, day-to-day variability.

ranges in pharmaceutical analytical chemistry however often make it possible to use quasi-linear calibration functions over the limited concentration ranges to avoid calibration via higher polynomial functions.

Generally, selectivity, separation power and sensitivity (expressed as quantitation limit) are lower for TLC or HPTLC than for LC. For assays, measurement uncertainty and precision data from validation experiments are comparable, whereas for quantification of impurities and related substances, the higher variability and measurement uncertainty and the resulting higher quantitation limit normally make HPLC the preferred analytical technique. TLC may be the method of choice if the detection characteristics of the analyte does not encourage the use of LC, e.g. for phospholipids that require light-scattering detection in LC.

Table 4 gives a rough estimation of selected performance characteristics of TLC–HPTLC.

Features and Advantages of TLC

There is a general shift away from TLC to HPLC for the control of impurities in new or revised pharmacopoeial monographs. Planar chromatography has a number of features and advantages (**Table 5**) so that, for some of the applications listed above, this technique must be considered to be at least an alternative to other chromatographic methods.

Actual Use of TLC in Pharmaceutical Analyses

As outlined in Table 3, TLC may theoretically be used for nearly every analytical task in the pharmaceutical industry. However, the general lower performance characteristics compared to LC on one hand and the special features (outlined in Table 5) on the other hand have resulted in some specifically traditional applications of TLC. These are identification, especially for port-of-entry testing, single container testing and for samples of natural origin like plant extracts or fermentation products. In its semiquantitative mode it is used for fast on-site testing. Applications in pharmacokinetic or bioavailability studies as well as quantitative determinations of impurities or related substances and assays of active pharmaceutical ingredients are still exceptions, mostly limited to cases where the matrix or the detection features of the compounds are to be determined, or the matrix excludes or limits the use of other chromatographic techniques.

Table 6 lists the main applications of TLC in pharmaceutical analysis today. For most of these applications, TLC is either mandatory or at least recommended by the respective pharmacopoeias and compendiums or encouraged by regulatory authorities.

Economic and Environmental Considerations

Most separations by TLC are performed in a normal-phase mode, using unmodified silica gel pre-coated layers, whereas HPLC in pharmaceutical analysis is nearly always used in the reversed-phase mode. Therefore, Table 6 lists predominantly examples where the straight-phase mode of TLC must be considered more appropriate than reversed-phase or examples where normal-phase TLC acts as a supple-

Table 5 Advantages and features of TLC/HPTLC

Simplicity of handling, easy to learn technically
Flexibility, short preparation time needed prior to analysis
Broad choice of mobile-phase systems
Numerous (> 1000) sensitive and selective reagents for detection and/or visualization
No obligation for elution of the analyte
Whole chromatographic information is stored on the plate and can be (re)evaluated
Detection and/or quantitation steps can easily be repeated using different parameters
Simultaneous yet independent analysis/samples of several samples and reference standards on one plate offers a high sample throughput and an increased reliability of results (in-system calibration)
Procedures are generally robust, allowing easy transfer and adoption
The single use of the plates offers the ability to handle crude, complex or dirty samples, even those with particulate impurities
Modular structure

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Table 6 Main applications of TLC in pharmaceutical analyses (qualitative, semiquantitative and quantitative)

Identity testing of raw materials, components and drugs in quality control, on-site or at port-of-entry
Single container identification if spectroscopic techniques (e.g. near infrared (NIR)) not suitable or available
Identity, purity testing and assay of herbal/vegetable material, extracts and drugs
Single, rapid on-site/port-of-entry testing for adulterated and faked drugs
Purity and related substance testing or assays if analyte has problematic detection characteristics (polyglycol derivatives, quaternary ammonium salts, phospholipids) and/or the materials require laborious and error-prone sample workup steps in other chromatographic techniques
Additional straight-phase technique parallel to RP-HPLC or other techniques for proving procedure selectivity in validation, for second assays of reference standards, in stability testing to prove that primary technique indicates stability and in forced degradation studies to give better mass balance

mentary or additional technique run in conjunction with reversed-phase HPLC (**Figure 2**).

There is a main field of application, however, where TLC and HPLC may be regarded as competing techniques. These are assays and content determinations of finished pharmaceutical products. HPLC has been the most popular analytical technique for these determinations for many years (and, like TLC, the most popular compendial technique for impurity and related substances testing) within the last few years. But papers dealing with TLC in pharmaceutical analysis are focusing more and more on final product assay and content testing, especially on content uniformity testing of tablets.

This application combines the technique's inherent advantages, its ability to accept complex or dirty sample matrices without requiring time-consuming sample clean-up steps, and its ability to run several samples on one plate in parallel and to be readily available without requiring system set-up and equilibration times.

In addition, TLC generally requires fewer solvents and chemicals than HPLC procedures.

Reports on the costs of performing multiple drug analyses conclude that cost reductions using TLC or HPTLC instead of LC (HPLC) can be significant. This is especially the case when many repetitive analyses have to be performed at one time and automated HPLC systems are not economic, as the total number of batches is too low or manufacturing is not evenly distributed throughout the year. If the obtainable separation performance parameters are considered sufficient, that is, if the TLC procedures have precisions within the required limits and a random standard deviation (RSD) of 2%, as required by the USP, then planar chromatography offers an alternative that costs less, is faster and has less impact on the environment. In addition, this type of TLC can in certain cases be performed even in hospitals, pharmacy laboratories or ports of entry, warehouses or

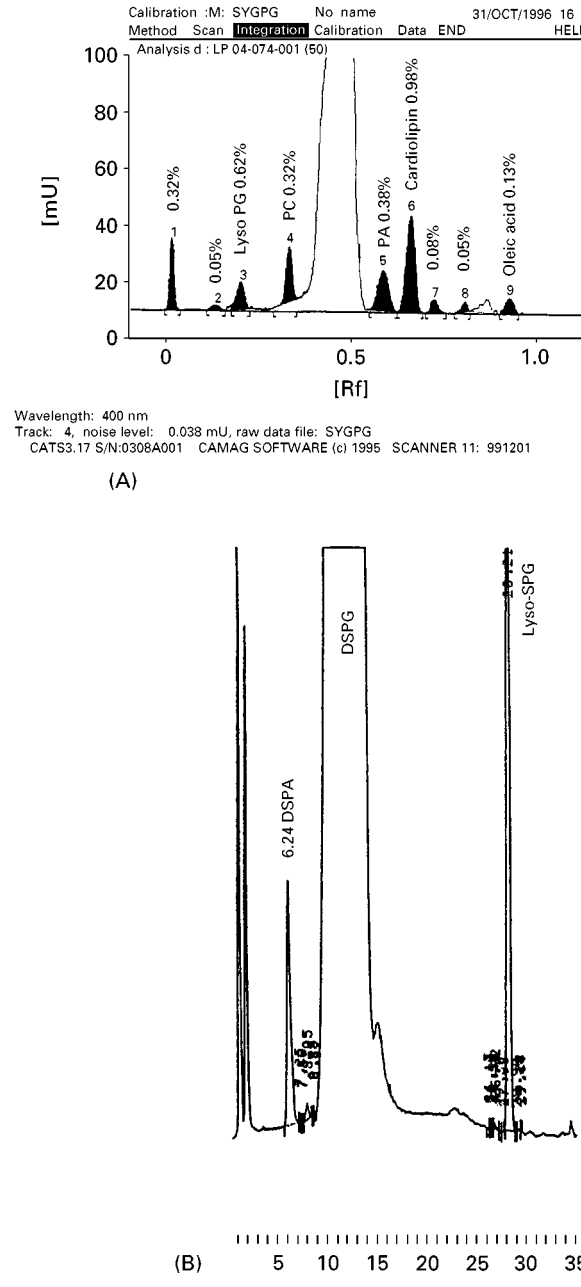

Figure 2 Comparison of HPLC and HPTLC analyses of phospholipids. (A) HPTLC, copper (II) sulfate/phosphoric acid derivatization and scanning densitometry. (B) HPLC, light-scattering detection. (A) Lyso PG, lyso phosphatidyl glycerol; PC, phosphatidyl choline; PA, phosphatidic acid; main component, 1-palmitoyl-2-oleyl-3-phosphatidyl glycerol; %, peak area compared to main component. (B) DSPA, phosphatidic acid; lyso LPG, lyso phosphatidyl glycerol; main component, 1,2-distearoylphosphatidyl glycerol (DSPG).

small control laboratories. However, although most of the published applications in the field have their origin not in the highly HPLC-oriented first world, the approach must not be considered to be only valuable and suitable for simple control laboratories with very limited assets.

Pharmaceutical analytical laboratories work under enormous economic and time pressure. Sample throughput has to be increased and lead times decreased, both without any consequence to the reliability of the results and at best without increase in personnel and operational costs. Therefore it must be recommended that TLC is considered as a possible replacement or substitute for compendial or noncompendial HPLC assays and content uniformity testing, especially for tablets.

Future Perspectives

A shift away from the traditional, semiquantitative TLC compendial purity testing of active pharmaceutical ingredients must be expected. Instead, a higher level quantitative TLC will be recommended and promoted by various national/international regulatory agencies for on-site and port-of-entry testing of pharmaceuticals. This development will be stimulated by the development of modern video imaging systems. Originally developed for documentation, modern closed-circuit device cameras are now combined with powerful software to collect the information stored on a plate in a very short time. Although not yet generally as precise and accurate as scanning densitometry, latest published results indicate that at least in the UV-region, video imaging can produce assay results that are equivalent to those derived by scanning densitometry. The lower price of video integration systems makes them the ultimate choice for rapid quantitative TLC applications.

The use of scanning densitometry will be limited to more delicate analytical tasks like stability or quality control testing procedures such as assays or content testing of finished pharmaceuticals, especially tablets, where TLC will expand because of its economical and environmental advantages, compared to HPTLC.

Highly sophisticated hyphenated techniques or combinations of TLC with spectrometric techniques such as FT-NIR, FT-IR, Raman or MS with different ionization mechanisms and MS-MS will be improved, but they will remain special technical solutions to special problems and must not be expected to become routine methods.

See also: **II/Chromatography: Thin-Layer Planar:** Densitometry and Image Analysis; Historical Development; Instrumentation; Layers; Mass Spectrometry; Modes of Development: Conventional; Modes of Development: Forced Flow, Overpressured Layer Chromatography and Centrifugal. **III/Pharmaceuticals:** Basic Drugs: Liquid Chromatography; Capillary Electrophoresis; Neutral and Acidic Drugs: Liquid Chromatography; **Thin-Layer Chromatography-Vibration Spectroscopy.**

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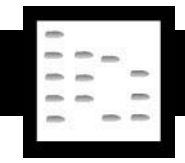

Gas Chromatography

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Phenolic compounds are extensively used in the chemical industry. Some simple phenolic species are known to be products of vascular plant metabolism, and also contribute to the polymeric structures of tannins and lignin. In addition to their use as intermediates in industry and in the production of dyes, plastics and pharmaceuticals, chlorophenols have